Review

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The impact of recent advances in genetics in understanding disease mechanisms underlying the long QT syndromes

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Abstract: Long QT syndrome refers to a characteristic abnormality of the electrocardiogram and it is associated with a form of ventricular tachycardia known as torsade-de-pointes and sudden arrhythmic death. It can occur as part of a hereditary syndrome or can be acquired usually because of drug administration. Here we review recent genetic, molecular and cellular discoveries and outline how they have furthered our understanding of this disease. Specifically we focus on compound mutations, genome wide association studies of QT interval, modifier genes and the therapeutic implications of this recent work.

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Keywords: cardiac arrhythmia; HERG; KCNQ1; long QT syndrome; potassium channel; SCN5A; sodium channel; sudden cardiac death.

Introduction

Sudden cardiac death (SCD) is a major health care problem and claims up to 300 000 lives per year in the USA alone (Deo and Albert, 2012). It occurs most commonly in older individuals with ischaemic heart disease however, SCD also occurs in younger patients under the age of 35. In this group the causes are more heterogeneous including a number of unique syndromes largely with a genetic basis (Deo and Albert, 2012). The hereditary long QT syndromes are one such entity and lethal arrhythmia is present in the absence of structural heart disease (Moss and Kass, 2005; Schwartz et al., 2012). The QT interval refers to a measurement made from the surface ECG and reflects the time taken for repolarisation of the cardiac action potential (Figure 1A). It is rate dependent with shortening at higher heart rates and thus is rate corrected using a variety of algorithms of which Bazett's correction is the one most commonly used [QTc=QT/sqrt(R-R)] though with poor justification (Karjalainen et al., 1994). By itself a prolonged QT interval is not harmful but it can lead to a particular form of ventricular tachycardia known as torsade de pointes and this can degenerate into ventricular fibrillation and death (Figure 1B). Hereditary and acquired forms of the disease are recognised with the latter particularly associated with administration of a wide range of different drugs including non-cardiac medications.

The incidence of the hereditary long QT syndrome is approximately 1 in 2000 births and two clinical syndromes are described (Schwartz et al., 2009). The first is Romano-Ward syndrome which is inherited in an autosomal dominant fashion and is relatively common (Ward, 1964; Romano, 1965). The rarer Jervell-Lange-Nielsen syndrome is an autosomal recessive disease and is additionally associated with hearing loss. It is also severer with a substantially prolonged QTc and cardiac arrest at a young age (Jervell and Lange-Nielsen, 1957). Classic genetic approaches in families with large pedigrees revealed the causative genes. Fifteen genetic loci and genes have been linked with hereditary long QT syndromes (Table 1). In most modern series a mutation is identified in 80% of patients. LQT1 and LQT2 evenly account for close to 90% of all gene positive cases with LQT3 accounting for 8% and the other causes being rare (Splawski et al., 2000). Indeed some of these have only been described in a single family or proband. It is worth noting that in two of the LQT syndromes, though they are very rare, they have a series of other disease manifestations. In Anderson's syndrome (LQT7) there are skeletal abnormalities and periodic paralysis in addition to long QT and malignant ventricular arrhythmias (Plaster et al., 2001; Tristani-Firouzi et al.,

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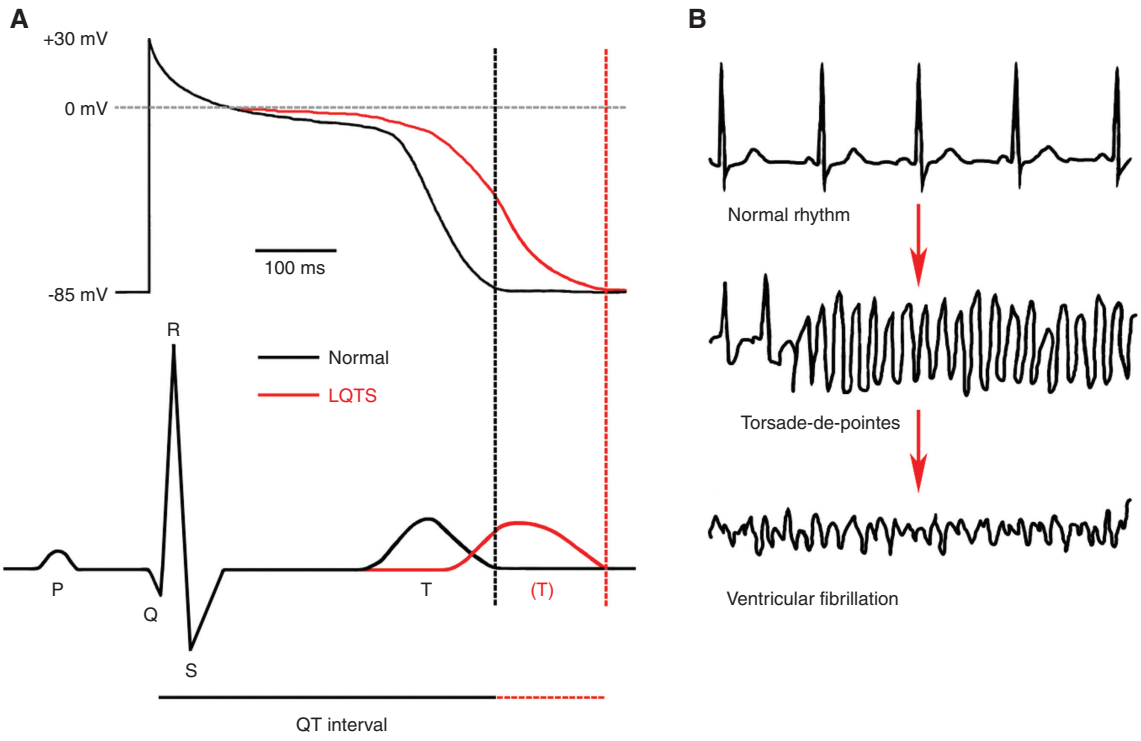

Figure 1: LQTS mutations lead to delayed ventricular myocyte repolarisation, a prolonged QT interval, and can under certain situations precipitate arrhythmic events.
(A) Mutations in the major LQTS causing genes (LQT1-15) act, in general, to result in either a delayed or reduced potassium efflux (red line). This delay in ventricular myocyte repolarisation is manifest on a surface electrocardiogram (ECG) as a prolonged QT interval. The 'T' wave on an ECG corresponds to the repolarisation phase of the heart. The end of the 'T' wave is indicated by the dashed line. When corrected for heart rate the QT interval is considered prolonged if >450 ms. (B) In LQTS patients a prolonged QT interval can act, alongside other triggers, to precipitate a particular characteristic type of arrhythmia called torsade de pointes (TdP). TdP can further degenerate into ventricular fibrillation, syncope and sudden death.

2002). In Timothy syndrome (LQT8) the extra-cardiac features include webbing of fingers and toes, autism, intermittent hypoglycaemia and immune impairment (Splawski et al., 2004).

The QT interval is related to the action potential duration and thus it was not surprising that the LQT genes encoded ion channels governing excitability. KCNQ1 (Kv7.1\KvLQT1) (LQT1) is a shaker like voltage-gated K^+ channel subunit that together with the β subunit KCNE1 (LQT5) underlies the I_{Ks} current (Barhanin et al., 1996; Sanguinetti et al., 1996b). HERG (KCNH2, Kv11.1) (LQT2) together with perhaps KCNE2 (LQT6) generates the I_{Kr} repolarising K^+ current (Sanguinetti et al., 1995; Trudeau et al., 1995; Abbott et al., 1999). SCN5A (LQT3) is the cardiac sodium current and CACN1C ($Ca_v1.2$, LQT8) the L-type calcium current (Wang et al., 1995). KCNJ2 (LQT7) underlies some if not all I_{K1} and is mutated in Anderson's syndrome (Tristani-Firouzi et al., 2002). All of these genes can lead to the Romano-Ward syndrome whilst only mutations in KCNQ1 (LQT1) and KCNE1 (LQT5) can lead to Jervell-Lange-Nielsen syndrome (Tyson et al., 1997).

There are other interesting cardiac clinical features. Cardiac arrest in LQT1 tends to be provoked by exercise or stress and swimming is a particular trigger. In LQT2 it is sudden often auditory events such as being woken from sleep by an alarm or an unexpected telephone call (Schwartz et al., 2001). In LQT3 arrhythmic events tend to occur during sleep. There are also mutations in SCN5A that can lead to both LQT3 and Brugada syndrome (Makita et al., 2008). Thus the hereditary long QT syndromes have traditionally been viewed as a classic monogenic genetic disorder with mutations in a number of different genes leading to the clinical disorder.

Disease mechanisms

Mutation type and location

As detailed above the majority of long QT syndrome cases are caused by mutations of the genes encoding the

Table 1: Long QT syndrome subtypes and the cardiac ion channel complexes and currents affected.

LQTS subtype	Gene	Locus	Protein	Ion channel affected	Frequency of cases (%)	Comments
Ion channels:						
LQT1 (JLNS/RWS)	KCNQ1	11p15.5	Kv7.1/KvLQT1	$I_{Ks}\downarrow$	40–45	I_{Ks} is enhanced by adrenergic stimulation
LQT2 (RWS)	KCNH2	7q35–36	Kv11.1/HERG	$I_{Kr}\downarrow$	30–35	Reduced repolarisation reserve
LQT3 (RWS)	SCN5A	3p21–p24	Nav1.5	$I_{Na\,(late)}\uparrow$	5–10	Gain-of-function, persistent late inward current
LQT7 (AS)	KCNJ2	17q23	Kir2.1	$I_{K1}\downarrow$	<1	Late phase 3–4 repolarisation affected
LQT8 (TS)	CACNA1C	12p13.3	Cav1.2	$I_{CaL}\uparrow$	Very rare	Gain-of-function
LQT13 (RWS)	KCNJ5	11q24.3	Kir3.4	$I_{KACh}\downarrow$	Very rare	Kir3.4 expression originally thought to be limited to the atria
Ion channel auxiliary subunits:						
LQT5 (JLNS/RWS)	KCNE1	21q22.1–22.2	MinK/IsK/KCNE1	$I_{Ks}\downarrow$	<1	May also affect I_{Kr}- KCNE1 may be promiscuous
LQT6 (RWS)	KCNE2	21q22.1	MiRP1/KCNE2	$I_{Kr}\downarrow$	<1	May also affect I_{Ks}- KCNE2 may be promiscuous
LQT10 (RWS)	SCN4B	11q23.3	Sodium channel beta subunit 4	$I_{Na}\uparrow$	Very rare	Gain-of-function
Ion channel interacting/modulating proteins:						
LQT4 (RWS)	ANK2	4q25–q27	Ankyrin B	Multiple$\downarrow$	<1	Loss-of-function of multiple channels
LQT9 (RWS)	CAV3	3p25	Caveolin-3	$I_{Na\,(late)}\uparrow$	<1	Other ion channels may also scaffold in caveolae
LQT11 (RWS)	AKAP9	7q21–q22	Yotiao	$I_{Ks}\downarrow$	Very rare	Modulates/translates PKA activation of I_{Ks}
LQT12 (RWS)	SNTA1	20q11.2	Syntrophin-alpha 1	$I_{Na\,(late)}\uparrow$	Very rare	Alters kinetic properties of I_{Na} resulting in a persistent late inward current
LQT14	CALM1	14q32.11	Calmodulin	Multiple?	<1	Disruption of calcium signalling – calmodulin also directly interacts with some channels including I_{Ks}
LQT15	CALM2	2p21	Calmodulin	Multiple?	<1	As above (CALM1)

References detailing the identification and characterisation of the 15 LQTS genes are provided in the main text. LQTS, Long QT syndrome; JLNS, Jervell and Lange-Nielsen syndrome; RWS, Romano-Ward syndrome; AS, Anderson's syndrome; TS, Timothy syndrome; I_{Ks}, slow component of the delayed potassium rectifier current; I_{Kr}, rapid component of the delayed potassium rectifier current; I_{Na}, cardiac fast sodium current; I_{K1}, cardiac inward potassium rectifier current; I_{CaL}, cardiac L-type calcium current; I_{KACh}, acetylcholine activated G-protein gated inward potassium rectifier channel.

outward potassium currents I_{Ks} and I_{Kr} carried by KCNQ1 and HERG (LQT1 and LQT2), respectively. In both proteins there are a large number of described mutations which are distributed throughout the N- and C-termini and transmembrane domains with some evidence of clustering around the pore (Splawski et al., 2000; Kapplinger et al., 2009). The majority of mutations are missense (~85%) with frame-shift and nonsense mutations being rarer. In the majority of cases with missense mutations an abnormal protein will be generated and then there are a number of potential mechanisms that could be operative. The mutant protein could traffic to the plasma membrane and when resident there either not function at all or have some abnormality in its normal gating. Alternatively there may be a cellular trafficking deficit: the protein may be unstable in the plasma membrane or be retained within the cell. There is substantial evidence for both mechanisms and for certain mutations multiple mechanisms can be operative (Sanguinetti et al., 1996a; Chouabe et al., 1997; Franqueza et al., 1999; Huang et al., 2001; Moss and Kass, 2005). There are correlations with mutation location and severity of disease for LQT1 and LQT2. In LQT1 and LQT2 mutations in the transmembrane regions of KCNQ1 and pore region of HERG, respectively have been shown to increase the risk of arrhythmia (Moss et al., 2002, 2007).

Defective channel function

Mutations in KCNQ1 and HERG (LQT1 and 2) can cause dysfunction by inhibiting the ability of these channels to open/gate properly. These defects result in a loss-of function and reduce the level of I_{Ks} or I_{Kr} current. In contrast, the sodium channel mutations in LQT3 are gain of function mutations and result in persistent inward sodium current. One of the first studied mutations was a three amino acid deletion (KPQ) in the III-IV linker region (Bennett et al., 1995): a region that is important for voltage dependent inactivation (Catterall, 2012). This mutation was subsequently engineered into the mouse genome and replicated many aspects of the human disease (Nuyens et al., 2001; Head et al., 2005). The study of a large number of other mutations confirmed changes in biophysical properties of the current engendered by the mutation led to excessive and persistent inward current as a general feature of the condition (Wang et al., 1995; Wang et al., 1996). In an analogous manner in Timothy syndrome the G406R mutation in CACN1C results in increased calcium current due to a loss of inactivation (Splawski et al., 2004).

Defective channel trafficking

The aberrant trafficking of channel complexes appears to play a major role in both LQT1 and LQT2 disease pathogenesis (Zhou et al., 1999; Ficker et al., 2000; Paulussen et al., 2002; Wilson et al., 2005; Dahimene et al., 2006). In fact, in LQT2 it has been suggested to be the most important mechanism (Anderson et al., 2006, 2014). Of the mutations in HERG or KCNQ1 that cause defective trafficking the vast majority result in retention of the channel protein in endoplasmic reticulum (ER) (Ficker et al., 2000; Wilson et al., 2005). In one case of LQT1 the Y111C mutation enhanced the degradation of the channel by the proteasome (Peroz et al., 2009).

Frameshifts, premature termination codons and nonsense mediated decay

Premature termination codon generating mutations may potentially result in the production of shorter truncated channel proteins. However, we are now more aware of nonsense mediated decay as a mechanism that can lead to RNA degradation of nonsense mutations prior to protein translation (Frischmeyer and Dietz, 1999). There is evidence for this with HERG though it has not been explicitly examined for KCNQ1 to our knowledge (Gong et al., 2007). The mutations will lead to haploin insufficiency in the heterozygous form and complete loss of function in the homozygous form. In LQT1 nonsense mutations have been associated with a lower arrhythmic risk when compared to missense mutations (Ruwald et al., 2015). Interestingly, frameshift mutations appear to have an equivalent risk to missense mutations, perhaps because some of these may be in-frame or may be less prone to nonsense mediated decay resulting in the production of mutant protein (Ruwald et al., 2015).

Additional disease mechanisms in RWS

There is another important factor to consider in dominant LQT syndromes. Potassium channels, such as KCNQ1, HERG and KCNJ2, are tetrameric proteins and thus can be subject to dominant negative effects (Herskowitz, 1987). This refers to the significant functional impairment that can arise from the co-expression of a normal and mutant allele. For example, if the inclusion of a single mutant allele in a potassium channel tetramer inactivates function then this will lead to loss of over 90% of current from binomial considerations of

the dominant negative effect in addition to the haploinsufficiency. Recessive LQT syndrome does occur as in JLNS and we and others have addressed the question of why the KCNQ1 and KCNE1 mutations do not have consequences in the carriers. It is notable that the KCNQ1 mutations are often nonsense. The assembly of potassium channels into tetramers is often determined by well-defined domains (Tinker, 2002). In the case of KCNQ1 the domain is present in the C-terminus and thus premature truncation of the protein before this point would mean that the mutant protein is unable to interact with the wild-type channel (Chouabe et al., 1997; Schmitt et al., 2000; Huang et al., 2001). Even missense mutations such as R594Q were proposed to act in this fashion (Schmitt et al., 2000; Huang et al., 2001). However, as we discussed above nonsense mediated decay is likely to be the predominant mechanism with nonsense mutations and this would lead to pure haploinsufficiency in heterozygotic mutation carriers. This in our view is the more probable explanation though this needs to be examined experimentally.

Auxiliary subunits

In addition to the main pore forming subunit there are also mutations in the auxiliary subunits KCNE1 and KCNE2. KCNE1 profoundly modifies the function of KCNQ1 and is well accepted as contributing with KCNQ1 to the molecular equivalent of I_{Ks} (Barhanin et al., 1996; Sanguinetti et al., 1996b). KCNE2 has been suggested to perform an analogous modulatory role for HERG but this is much more controversial (Abbott et al., 1999). We looked at the behaviour of a number of LQT5 causing KCNE1 mutants and found a mixture of mechanisms including abnormal gating and impaired assembly (Harmer et al., 2010). Trafficking impairment was relatively modest for the mutants we studied but has been reported by other groups (Krumerman et al., 2004). This said there is good evidence that the KCNE subunits may be much more promiscuous in their interactions with ion channels and this has largely not been factored into discussions of the potential pathogenic mechanism (McDonald et al., 1997; Decher et al., 2003; Wu et al., 2006; Abbott et al., 2007; Roepke et al., 2008; Nof et al., 2011). There is also evidence that mutations in a sodium channel subunit SCN4B might cause severe long QT syndrome (LQT10) (Medeiros-Domingo et al., 2007). Expression of the SCN4B missense mutation, L179F, with SCN5A led to a persistent inward sodium current as in LQT3.

Human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) models of LQTS

Cardiomyocytes derived from human induced pluripotent stem cells (iPSCs) is a rapidly emerging technology that was first used in 2009 (Zhang et al., 2009). Soon after, Moretti and colleagues published the first hiPSC-CM model of LQTS (Moretti et al., 2010). Fibroblasts from two LQT1 patients carrying the KCNQ1-G569A (R190Q) mutation and two healthy controls were reprogrammed to generate iPSCs. These iPSCs were then differentiated towards cardiomyocytes and their electrophysiological properties analysed. The action potentials (APs) of both atrial-like and ventricular-like hiPSC-CMs were found to be prolonged and the peak tail current density of the I_{Ks} current was reduced by 75% in cells carrying the KCNQ1-G569A mutation when compared to the healthy controls. In 2011, two groups published iPSC models of LQT2 and the hiPSC-CMs generated from LQT2 patients had prolonged APs and reduced I_{Kr} current density (Itzhaki et al., 2011; Matsa et al., 2011). The cardiac features of LQT8 (Timothy syndrome) were also modelled in the same year by Yazawa and colleagues (Yazawa et al., 2011). hiPSC-CMs with the CACNA1C-G1216A (G406R) mutation had larger L-type calcium currents and delayed calcium channel inactivation. The alterations in calcium channel properties also led to abnormal intracellular calcium handling, prolonged calcium transients, and ventricular-like action potentials that were three times longer than those seen for hiPSC-CMs from unrelated controls (Yazawa et al., 2011). An LQT3 causing mutation (SCNA5-5387_5389insTGA (1795insD) this mutation is also associated with the Brugada syndrome) was studied using iPSC technology in 2012 (Davis et al., 2012). The LQT3 hiPSC-CMs had prolonged APs and increased levels of persistent 'late' sodium current when compared to control cells from an unaffected control patient (Davis et al., 2012). In general, iPSC-CM based modelling of LQTS has largely recapitulated the expected electrophysiological phenotypes based on patient ECG morphology, animal models, and prior heterologous cell expression studies (see Hoekstra et al., 2012).

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Although iPSC-CM based models of LQTS are exciting there exist some concerns about their ability to fully model the electrophysiological consequences of disease causing mutations. In particular, iPSC-CMs are immature in their nature, both electrophysiologically and morphologically (Knollmann, 2013). Electrophysiologically, the APs produced from hiPSC-CMs can be very heterogeneous in nature and these cells lack robust expression of the inward rectifying current I_{K1}. The lack of I_{K1} is of particular concern as it leads to depolarised resting membrane

potentials, increased spontaneous activity, and a greater dependence on other currents for repolarisation (in particular I_{Kr}) (Doss et al., 2012; Lieu et al., 2013). Approaches that lead to the maturation of iPSC-CMs towards a more adult phenotype are actively being pursued. Recently, in an effort to counter the heterogenous nature of the APs produced by hiPSC-CMs gene editing has been used to generate isogenic stem-cell 'pairs' for LQT2 and LQT3 disease modelling (Bellin et al., 2013; Zhang et al., 2014). This approach allows researchers to investigate the effect of a particular mutation under the same cellular genetic background. Isogenic stem-cell 'pairs' should also offer exciting opportunities for studying the role played by complex genetics and LQTS disease modifiers such as single nucleotide polymorphisms (see below and Munroe and Tinker, 2015). The human and patient specific nature of iPSC-CM technology provides a great deal of scope for developing therapies to better understand, treat and manage LQTS. Indeed, as an example, a number of different LQTS subtype iPSC models have already been screened for their propensity to drug-induced arrhythmia (Liang et al., 2013). Approaches to rescue/reverse the LQT2 disease phenotype, such as pharmacological based rescue of defective channel trafficking (Mehta et al., 2014) and small interfering RNA (siRNA) mediated 'knockdown' of mutant hERG allele expression (Matsa et al., 2014), appear promising.

Physiological triggers of arrhythmia in LQTS

As described above it is clear that arrhythmia can be provoked by exercise in particular swimming in LQT1 and sudden arousal in LQT2 (Schwartz et al., 2001). That swimming in particular should provoke arrhythmia in LQT1 is interesting and suggests that both sympathetic and parasympathetic coactivation is especially proarrhythmic (Shattock and Tipton, 2012). The common thread linking these is activation of the adrenergic system. During exercise the action potential and QT interval shorten with increasing heart rate and a key factor in this is the increase in I_{Ks} current. This occurs for two reasons. The first is that current accumulates at high rates as deactivation kinetics are slow and at faster rates, between each action potential, the channel fails to fully deactivate (Stengl et al., 2003). A second reason is that increased sympathetic drive during exercise activates β-adrenergic receptors increasing the current levels. This regulation is probably much more important than is commonly appreciated as I_{Ks} blocking drugs have modest effects on action potential duration in isolated ventricular myocytes but the effects are much more pronounced on the QT interval *in vivo* (Volders et al., 2003). It should be borne in mind that in general there is a substantial amount of 'repolarisation reserve' due to the presence of I_{Kr} and I_{K1} in addition to I_{Ks} in cardiac ventricular myocytes. This likely explains in general terms the variable penetrance of disease causing mutations and variable effects of QTc prolonging drugs (Lengyel et al., 2004; Jost et al., 2005; Nagy et al., 2009).

The effects of sympathetic nervous drive on I_{Ks} is a classic pathway mediated via protein kinase A activation and direct channel phosphorylation on the N-terminus at S27 (Marx et al., 2002). There have been reports of particular mutations in LQT1 and LQT5 that impair PKA dependent phosphorylation and activation of the channel (Kurokawa et al., 2003; Heijman et al., 2012; Bartos et al., 2014). The A341V KCNQ1 mutation is particularly interesting. It is a founder mutation in large South African pedigrees and is generally very severe in clinical expression (Brink et al., 2005). However, when studied initially in heterologous systems it was found to have relatively mild functional impairment (Wang et al., 1999). This paradox was resolved when the mutation was found to lead to severely abrogated PKA dependent regulation that also occurred in heteromultimeric channel populations mimicking RWS (Heijman et al., 2012). Furthermore, the activation requires association of KCNQ1 with KCNE1 and the microtubule network and is dependent on a protein kinase A anchoring protein (AKAP) yotiao\AKAP9 (Marx et al., 2002; Kurokawa et al., 2003; Nicolas et al., 2008). A specific phosphodiesterase may also be part of this complex and yotiao\AKAP9 itself needs to be phosphorylated by PKA (Chen et al., 2005; Terrenoire et al., 2009). Mutations in yotiao\AKAP9 have been associated with the long QT syndrome (LQT11) but only in a single family to our knowledge (Chen et al., 2007). Other cardiac K^+ currents such as HERG can also be regulated by protein kinases but the physiological significance of this is less clear (Sroubek and McDonald, 2011; Shu et al., 2013). It is not clear as to why LQTS patients are predisposed to torsade-de-pointes as opposed to other forms of ventricular tachycardia. It seems the generation of early afterdepolarisations is important with this occurring particularly in the endo and mid-myocardium promoting re-entry (Akar et al., 2002).

Rare causes of LQTS

There are a series of other causes of LQT syndrome which are rare. These include mutations in Ankyrin (LQT4) (Mohler et al., 2003), caveolin-3 (LQT9) (Vatta et al., 2006),

α-1-syntrophin (LQT12) (Wu et al., 2008; Ueda et al., 2008), KCNJ5 (LQT13) (Wang et al., 2013) and calmodulin 1 and 2 (LQT14 and LQT15) (Crotti et al., 2013). Plausible cases can be made for these proteins in modulating cardiac ventricular excitability and being potentially arrhythmogenic however, the pedigrees and number of families are often limited.

Summary

Thus there has been substantial work on the mechanism by which the known mutations potentially influence myocardial ventricular excitability. However, recently there have been some interesting genetic findings that modify our understanding of disease pathogenesis and manifestations in the individual. We consider compound mutations, genome wide association studies on the QT interval and modifier genes.

Compound mutations increase arrhythmic risk

In the inherited long QT syndromes compound mutations are common with a frequency of close to 10% and intriguingly are associated with a more malignant course and higher arrhythmia burden (Schwartz et al., 2003; Westenskow et al., 2004; Tester et al., 2005; Itoh et al., 2010; Giudicessi and Ackerman, 2013). They can occur within the same gene for example two different mutations on different alleles in the same gene or two mutations in different gene, e.g. KCNQ1 and KCNH2. Westenskow and colleagues investigated the first scenario (Westenskow et al., 2004). When a single mutation was expressed in a heterozygous manner recapitulating a potential RWS phenotype, they found only a small dominant negative effect. However, when expressed in compound form there was a striking reduction in I_{Ks} current density. The conclusion was that the severe clinical phenotype was accounted for by the additive effects of the single mutations on the I_{Ks} current (Westenskow et al., 2004). We have recently investigated this issue and asked are there any other pathogenic factors that may account for the pronounced disease manifestations (Harmer et al., 2014). Our study showed that failure of membrane delivery of KCNQ1 contributed significantly to the disease mechanism. Specifically, we identified a class of mutation that are severely retained but do not have a dominant negative effect on the trafficking of wild-type KCNQ1. For the combinations A178T/K422fs39X, T391I/Q530X and A525T/R518X there was an additive effect impairing channel trafficking as well as other functional effects that would exacerbate the phenotype (Harmer et al., 2014).

For the large majority of compound heterozygous KCNQ1 mutations reported, the probands present with autosomal recessive long QT syndrome and their hearing is normal. However, this is not the case in all families and there are situations where deafness occurs with two different KCNQ1 mutations present on different alleles (Giudicessi and Ackerman, 2013). There appears to be threshold effect where preservation of a tenth of the current is sufficient to rescue hearing. For example, in consanguineous Arabian families a homozygous recessive mutation was identified in the first intron in a splice acceptor site. This led to a significantly reduced but not totally absent KCNQ1 transcript (Bhuiyan et al., 2008). This idea is in keeping with our recent study. The compound effects of A525T/R518X and A178T/K422fs39X lead to ~85% loss of current but the mutations do not result in deafness (Harmer et al., 2014).

Complex genetics

At the population level the QT interval is a heritable trait and as such is amenable to the analysis of its genetic architecture using genome wide association studies (GWAS). The imputation of a particular gene as being causative in the trait is more problematic than in classic monogenic disorders (Munroe and Tinker, 2015). The linked loci often contain signals that are in introns or large intergenic regions and in linkage disequilibrium with other genes. However, a number of such studies have been undertaken and the results are informative. In some cases the loci clearly implicated known genes encoding proteins involved in cardiac repolarisation and in the hereditary long QT syndromes (Newton-Cheh et al., 2009; Pfeufer et al., 2009; Arking et al., 2014). In general, the effect size of a locus is relatively small and is about 3–5 ms. The most strongly associated signals were related to the nitric oxide synthase adaptor protein (NOS1AP) (Arking et al., 2006; Newton-Cheh et al., 2009; Pfeufer et al., 2009; Arking et al., 2014). At the time of the initial discovery it was unknown as to the role of NOS1AP in cardiac repolarisation. Functional studies showed that together with nitric oxide synthase it was a potential regulator of calcium channel function (Chang et al., 2008). Specifically the protein complex inhibited L-type calcium function shortening repolarisation. Kapoor and colleagues identified the potential causative SNP as a

noncoding polymorphism within an enhancer region of the NOS1AP leading to increased expression of the transcript (Kapoor et al., 2014). Furthermore, they showed that NOS1AP localised to the intercalated disc and that overexpression of NOS1AP protein in neonatal rat ventricular myocytes shortened the action potential and also perhaps surprisingly increased conduction velocity. A recent study of over 75,000 individuals identified loci particularly associated with myocyte calcium handling (Arking et al., 2014). Finally, the functional study of the ring finger protein, RNF207, another protein associated in GWAS for QT interval showed that it interacted with HERG (Roder et al., 2014). Overexpression of RNF207 led to an increase of HERG protein, membrane localisation and currents some of which was also dependent on coexpression of heat shock protein 70.

It has been appreciated for a number of years that particularly in Romano-Ward syndrome the penetrance of the disease is highly variable in mutation carriers (Priori et al., 1999). The explanation for this is still obscure but there have been some recent developments. The idea of variable 'repolarisation reserve' in different individuals has been used to explain why some develop drug induced long QT (Sesti et al., 2000). A similar concept could apply in the hereditary long QT syndrome but the practicalities of finding sufficiently large pedigrees meant this was difficult to explore experimentally. The convergence of study of the large family pedigrees carrying the KCNQ1 A341V mutation and candidate loci identified in GWAS enabled the design of credible experiments. This has enabled the identification of loci linked to NOS1AP and AKAP9 as potential modifier genes that can affect penetrance (Tomas et al., 2010; de Villiers et al., 2014). There are also suggestions that the KCNE1 D85N polymorphic variant may be more common in LQT syndrome probands (Nishio et al., 2009). Polymorphisms in the 3′ untranslated region of KCNQ1 also appear to modulate disease severity in patients with LQT1 (Amin et al., 2012). This study identified three SNPs that modulate disease severity and provides evidence that they modulate the expression of the allele (e.g. mutant or wild-type) on which they reside and therefore alter the balance of wild-type to mutant allele expression (Amin et al., 2012). Very recently SNPs that have been previously linked to QT-interval (as discussed earlier), (NOS1AP SNPs and a common KCNQ1 SNP) have also been identified as modifiers of disease severity in LQT2 patients (Kolder et al., 2015). Interestingly, the effects of these SNPs on the QT interval are larger in the LQT2 patients than the normal population and this is presumably due to their already reduced repolarisation reserve (Kolder et al., 2015).

Genetics and drug-induced long QT syndrome

A wide variety of different pharmacophores, including agents developed for diseases outside the cardiac arena, can prolong the QT interval and precipitate torsade-de-pointes and this presents a significant challenge for the pharmaceutical industry (Witchel and Hancox, 2000; Fermini and Fossa, 2003). The agents seem share a common mechanism of action namely blocking of the HERG channel (Sanguinetti et al., 1995). HERG uniquely seems to possess an accommodating binding pocket that allows interaction with many different chemical structures (Mitcheson et al., 2000). Despite the advances in ion channel structure determination we still do not have a high resolution structure of HERG to analyse this fully at the molecular level. Currently, there has been some success in the use of predictive tools based on quantitative structure-activity relationships that assess a compound's potential for HERG block (Aronov, 2008; Nikolov et al., 2014). The occurrence of drug induced long QT and torsade-de-pointes with a given drug is relatively rare and it is not clear why only some individuals develop the problem. In some cases the precipitant might be a known event such as inter-current illness leading to a fall in serum K^+ concentration or co-administration of another interacting drug. However, even with this taken into account specific individuals seem predisposed to drug induced long QT. The first idea was that this may be a forme-fruste or concealed form of hereditary LQTS revealed by drug administration. However, whilst it is possible to identify individual cases where this is the probable explanation, on balance the data seem to indicate that this is rare (probably 10% or less of cases) (Napolitano et al., 2000; Yang et al., 2002; Paulussen et al., 2004). A more likely scenario from recent studies is that the genes underlying population variation in QT account for the predisposition. For example, single nucleotide polymorphisms in the NOS1AP locus was increased in prevalence in a group of individuals with drug induced long QT (Jamshidi et al., 2012).

Clinical implications

How does improved knowledge of the genetic and molecular basis of long QT syndrome and attendant mechanisms impact on patient management? The mainstays of management are β blockers, implantable cardiac defibrillators and left cervical sympathectomy (Schwartz et al., 2012). There is evidence that implantable cardiac defibrillators

are overused with the attendant complications from a combination of over diagnosis and over treatment (Gaba et al., 2015). In the latest European Society of Cardiology guidelines genetic screening can help in algorithms for both diagnosis and management (Priori et al., 2015). Specifically, the identification of a particular mutation as causative in a patient means that a family can be screened and if carriers but asymptomatic given general advice to avoid certain drugs and environmental triggers (Schwartz et al., 2001). There has been interest in gene specific therapy, for example sodium channel blockers such as ranolazine in LQT3, and such studies are ongoing (Moss et al., 2008). The use of genetic information is likely to increase as next generation DNA sequencing technologies are brought to bear in clinical diagnostic services (Li et al., 2013).

An appreciation of the pathogenesis of the mutations involved in genetic disease has led to some promising approaches in cystic fibrosis. VX-770 activates the cystic fibrosis transmembrane conductance regulator (CFTR) and improves the function of specific mutant channels (G551D) which traffic normally but are unable to gate appropriately. This agent has been shown to be effective in patients improving a number of markers of disease progression (Ramsey et al., 2011). However, in the majority of cases of cystic fibrosis the CFTR channel contains a deletion of phenylalanine at position 508 resulting in retention in the endoplasmic reticulum and degradation (Ward et al., 1995; Seibert et al., 1997). Critically, if the channel is allowed to escape to the plasma membrane then it can form an adequate chloride conductance (Drumm et al., 1991). Chaperone like drugs have been developed to do this (VX-809) and in patient trials improve clinical parameters when given in combination with VX-770 (Wainwright et al., 2015). The important conclusion from these studies is that it may be possible to design mutation specific agents that ameliorate the underlying defect. In LQT2 there are data that the modulation of trafficking by E-4031 and ranolazine might be efficacious for a significant number of different mutations (Smith et al., 2013; Anderson et al., 2014). E-4031 is a HERG blocker but has a relatively short half-life and the effects on trafficking are much more prolonged than the blocking action (Smith et al., 2013). This is highly speculative yet in the long QT syndromes but illustrates that such a strategy may be feasible. Indeed, novel and specific channel KCNQ1 openers have been identified (Mattmann et al., 2012).

Another molecular approach is the promotion of read-through of nonsense mutations. This occurs with a number of aminoglycosides but newer agents have been developed that are better tolerated by patients (Welch et al., 2007). PTC124 is in development for Duchenne muscular dystrophy and cystic fibrosis though there are some doubts as to its efficacy (Kerem et al., 2008; Auld et al., 2009; McElroy et al., 2013; Mullard, 2014). This approach has been explored for LQT2 and sodium channel nonsense mutations (Yao et al., 2009; Teng et al., 2009) and we have also examined such a strategy for LQT1 and found that whilst full-length channel expression occurred there were also alterations in the biophysical properties of the resultant channels presumably resulting from the replacement of the stop codon with a harmful amino acid (Harmer et al., 2012).

Conclusions

There have been substantial advances in the genetics and underlying disease mechanisms of the long QT syndrome over the last 10 years. The use of findings from genome wide association studies especially has enabled some understanding of modifier genes responsible for variable penetrance and predisposition in the drug induced long QT syndrome. In the future the understanding of the pathogenic mechanism may enable mutation specific personalised therapy for patients addressing the underlying cellular defects.

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Q3: Please check and confirm the acknowledgment and conflict of interest statement section

Conflict of interest statement: The authors have no conflicts of interest to declare.

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